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Molecular Study of Sex Steroid Receptor Gene Expression in Human Colon and in Colorectal Carcinomas

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Background: Sex steroid hormones influence function of the human gastrointestinal tract. Although the specific receptor proteins have been identified in surgical specimens of both intestinal mucosa and colorectal carcinomas, it is still unknown whether they are expressed in intestinal epithelial cells.

Methods: Expression of androgen receptor (AR) protein and estrogen receptor (ER) protein was studied by Scatchard analysis and ELISA (for ER only) in surgical specimens of normal-appearing mucosa, colorectal carcinomas, isolated colonocytes, and human colorectal carcinoma cell lines. Northern analysis was applied to identify the appropriate mRNAs, followed by the sensitive technique of reverse transcription-polymerase-chain-reaction (RT-PCR).

Results: AR protein was identified in all surgical specimens analyzed and ER protein in 10 out of 13 normal-appearing mucosa specimens and 4 out of 7 colorectal carcinomas. The receptor proteins were not found in isolated colonocytes or in the transformed cell lines. RT-PCR confirmed that none of the isolated normal colonocytes or transformed colorectal carcinoma-derived cells expressed these mRNAs. Intestinal smooth muscle cells and fibroblasts were found to express sex steroid receptor mRNAs.

Conclusions: Both receptors are present in human large intestine but are expressed in stromal cells and not in intestinal epithelial cells. We hypothesize that sex steroids may influence the function of colonocytes indirectly through stromal-epithelial interactions. J. Surg. Oncol. 64:3–11

KEY WORDS: **androgens; estrogens; receptors; RT-PCR; colorectal neoplasms**

INTRODUCTION

Human large intestine and colorectal carcinomas were treated as hormonally independent tissues until recent experimental and clinical studies indicated that steroid hormones, retinoids, vitamin D3, epidermal growth factor, somatostatin, somatomedin, gastrin, and transforming growth factor alpha and beta may influence functions of epithelial cells in the large intestine [1–10]. Furthermore, it was reported that steroid hormones influenced mesenchymal cells in the human gastrointestinal tract [11,12].

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Cellular response to sex steroids is mediated directly through a specific superfamily of ligand-activated enhancer proteins, the steroid/thyroid hormone receptors [13,14], or indirectly by modifying other signalling pathways [2,15]. In the direct pathway, the activated receptor binds steroid hormone, which interacts with the steroid responsive element of genes, regulating their functional status [16]. Ligand-receptor-DNA interactions can be modified by polymerization, localization of steroid responsive elements with respect to other enhancing elements, transcriptional factors, oncoproteins, and mutations [17]. Consequently, even though the proliferation of some cells is modulated by sex steroids, cells that are not steroid-dependent nonetheless may contain receptors and, presumably, have particular steroid-responsive genes [18].

The presence of sex steroid receptors has been demonstrated in most normal and transformed cells of mesenchymal as well as epithelial origin [19]. Notably, many human breast cancer cells, endometrial cancer cells, and prostate cancer cells are steroid dependent in vitro and in vivo [20,21]. Qualitative analysis based on a one-point binding assay has identified estrogen receptors in human colorectal cancer tissue [22]. Sex steroid receptors in human colorectal carcinomas also have been determined quantitatively by Scatchard analysis [23], 16 out of 23 colorectal carcinomas being AR-positive and 30% of tumors being estrogen receptor (ER) positive. Additional studies have confirmed androgen receptor (AR) to be present in the majority of analyzed colorectal carcinomas, with ER identified in $<$50% of cases [3,24–28]. Some investigators, however, have reported negative results [29,30].

Because, as discussed above, studies of sex steroid receptors have been contradictory and only have been based on bulk surgical specimens, the current study was undertaken using colonocytes isolated from sigmoid mucosa and colorectal carcinoma-derived cell lines. Scatchard analysis confirmed the receptors were present in bulk surgical specimens, and molecular analysis of isolated normal colonocytes and transformed colonocytes (Northern analysis and RT-PCR) were used to determine which cells of the intestinal mucosa specifically expressed the receptors.

MATERIALS AND METHODS

Patient Population and Specimen Processing

Surgical specimens were obtained from two sets of patients, one being used for Scatchard analysis at the tissue level (carried out in Poland) and the other for studies at the cellular and molecular levels (carried out in the United States). The two studies are hereafter referred to as the "tissue study" and the "cellular/molecular study," respectively. For the tissue study, samples were obtained from seven cancer patients who underwent elective colorectal cancer surgery. Control specimens were obtained from four patients undergoing elective surgery for nonmalignant colorectal diseases. All the cancer patients were female and with one exception were postmenopausal. For the cellular/molecular study, 13 patients with nonhereditary colorectal carcinoma were studied.

The surgical specimens were sampled by the following schema. For the tissue study, a sample was obtained from each of the seven tumors, and from four of the specimens a sample of normal-appearing mucosa was obtained some 2–4 cm from the tumor. In three cases a sample of normal-appearing mucosa was obtained 10–15 cm from the tumor. Six normal-appearing mucosal samples were obtained from the four control patients. All the specimens were immediately immersed in ice-cold saline in the operating room. Mucosal samples were stripped from the underlying smooth muscle layer and frozen in liquid nitrogen within 15 minutes of removal from the patient. All samples were analyzed within a few weeks of collection. Parallel samples were collected for histopathology, which showed three adenocarcinomas at Dukes stage B and 4 adenocarcinomas at Dukes stage C, all well differentiated. None of the normal-appearing mucosal samples contained histopathological changes typical of dysplasia or neoplasia.

For the cellular/molecular study, normal-appearing mucosa was taken from jejunum (1 specimen), ileum (1 specimen), female cecum (2 specimens), and male cecum (1 specimen), female sigmoid (1 specimen), and male sigmoid (7 specimens) of nonhereditary rectal cancer patients.

Cell Culture and Isolation of Colonocytes

Established colorectal carcinoma cell lines, Caco 2, Colo 205, DLD 1, HT 29, SW 480, SW620, SW837, SW1463, representing different stages of the disease, human colonic fibroblasts CD-18Co, human intestinal smooth muscle cells HISM, LNCaP prostatic carcinoma cells (controls for both AR protein and AR mRNA) [31], and MCF-7 breast carcinoma cells (controls for both ER protein and ER mRNA) [32] were purchased from the American Type Culture Collection (Rockville, MD). Cancer cells were cultured until confluent in RPMI-1640 medium (Gibco BRL, Gaithersburg, MD) without phenol red [33]. Fibroblasts were cultured in Dulbeco's modified medium. Smooth muscle cells were cultured in modified Eagle medium. All media contained 10% heat-inactivated fetal calf serum (Gibco BRL).

For colonocyte isolation, mucosa was separated mechanically from the smooth muscle layer and cut into pieces 5×5 mm. The pieces were washed 30 minutes in DTT/RPMI-1640 solution (1.5 mg/ml) at 37°C with shaking. The mucosa pieces were incubated three times for 30 minutes each digestion with Dispase (Boehringer, Mannheim, Germany) dissolved in RPMI-1640 medium (3 mg/ml) with vigorous shaking (225 rpm). To remove

red blood cells, the cell suspension was incubated 15 minutes at room temperature in hypotonic solution (7 mg NH_4CO_3) and 700 mg NH_4Cl in 100 ml H_2O) and washed twice in cold PBS. Quality and viability of the intestinal epithelial cells were checked by light microscopy. The suspension contained 97% living cells as demonstrated by exclusion of Trypan Blue. The cells were easily identified as epithelial cells on the basis of morphology and size. No red blood cells were found. Also, stromal cells were not present except single lymphocytes (1.8% ± 0.3%) as demonstrated by indirect immunostaining of the air-dried, alcohol-fixed cellular smear using antikeratin monoclonal antibody AE1/AE3 (Boehringer), polyclonal antibodies directed to a desmin and a vimentin (Dako, Denmark), and a monoclonal antibody directed to a leukocyte common antigen CD45RB (Dako, Carpinteria, CA) [34].

Scatchard Analysis of Tissues

Ligands. [6,7-3 H] methyltrienolone (55.5 Ci/mmol), [6,7-3 H] estradiol (85.0 Ci/mmol), as well as unlabeled methyltrienolone, diethylstilbestrol and dexamethasone were purchased from New England Nuclear (Boston, MA). To avoid nonspecific binding of steroid, which leads to nonlinearization of Scatchard plots [35], radiolabeled methyltrienolone was used as the AR ligand instead of testosterone or dihydrotestosterone because this ligand does not react with blood proteins and has a high affinity to the receptor. Charcoal (Norit A) was purchased from Sigma Chemical Co. (St. Louis, MO). Prior to use, the charcoal was repeatedly washed with glass-distilled water to remove fine particles by decantation and finally with methanol. The radioactive steroids were repeatedly purified on silica gel thin layer plates to maintain their radiopurity above 99%. Dextran T 70 (M 70,000) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were analytical reagent grade and were used without further purification as obtained from Sigma or from Merck A.G. (Darmstadt, Germany).

Preparation of cytosol. Tumor tissue was homogenized mechanically in liquid nitrogen to a powder. The homogenized tissue was diluted in two volumes of buffer (50 mM Tris-HCl, pH 7.4, 23°C, 1 mM EDTA, 0.1 mM DTT, 10% (v/v) glycerol) and centrifuged at 105,000 *g* for 60 minutes. The pellet was frozen to −20°C for subsequent determination of DNA.

Ligand-receptor binding assay. Cytosol (0.1 ml) aliquots were added to tubes containing a 0.1 ml buffer solution of ^{3}H-labeled steroid at different concentrations from 0.1–5 nM (final concentrations) in the absence or presence of a 100-fold excess of unlabeled steroid to determine total and nonspecific binding, respectively. The final concentration of ethanol was below 1% (v/v). The incubations were carried out at 0°C–4°C for 16–20 hours. At the end of this period, 0.5 ml dextran-coated charcoal was added [0.5% (w/v) charcoal and 0.05% (w/v) Dextran T 70 in the homogenization buffer], and after vigorous mixing the incubations were continued for 20 minutes. After centrifugation at 1,000 *g* for 5 minutes, 0.5 ml supernatant fluid was taken for liquid scintillation counting. The total radioactivity was determined from tubes containing the same concentrations of steroids, except buffer was substituted for the cytosol and dextran-coated charcoal suspension. All incubations were performed in duplicate.

DNA measurement. DNA in the pellets was quantified according to Burton [36] as modified by Giles and Myers [37]. DNA samples at three dilutions were analyzed in duplicate, and unless the standard curve and the dilution series of the samples were parallel, the assay was repeated. The coefficient of variation within a set of determinations on a given sample of DNA was accepted when below 5%, and the mean value of such a set was used as the DNA concentration.

Statistical analysis. The calculation of binding parameters was performed according to Scatchard [38]. Nonspecific binding was subtracted according to Chamness and McGuire [39], using a statistical program written in Turbo Basic in the Institute of Animal Physiology and Nutrition, Polish Academy of Sciences. Unless the slope of the Scatchard plot was different from zero at the 5% level, the results were not interpreted. All statistical formulas used can be found elsewhere [40].

Scatchard Analysis and ELISA Assay for Normal and Transformed Colonocytes

The colonocytes isolated from the sigmoid specimen and two colorectal carcinoma cell lines, DLD-1 and HT-29 were analyzed by both Scatchard method (AR, ER) as described above and by commercially available ELISA assay kit (ER) (Abbott ER-EIA Monoclonal Kit, Abbott Laboratories).

Northern Analysis

Total RNA was isolated by the guanidinum thiocyanate-phenol-chloroform extraction described by Chomczynski and Sacchi [41]. Poly A(+) RNA was isolated as described by Sambrook et al. [42]. Approximately 30 μg of total RNA and 10 μg of poly A(+) RNA isolated from each case were separated by electrophoresis through 1% agarose gels containing 0.66 M formaldehyde and blotted overnight onto nylon membranes (Magna Graph, MSI, Westboro, MA) in 10× SSC buffer.

To identify AR mRNA, specific probe, 1.4 kb fragment of AR cDNA, coding ligand binding domain of the receptor, was prepared by digestion of full-length AR cDNA with restriction enzymes Bam HI and Hind III. Similarly, a specific probe for identification of ER mRNA, 0.7 kb fragment of ER cDNA, coding ligand binding domain,

was prepared by digestion of full-length ER cDNA with Eco RI and Hind III. With these probes, AR mRNA was expected to be expressed as a 10.5 kb band [43] and ER mRNA was expected to be expressed as a 4.2 kb band [44]. The specificity of the probes was verified by hybridization with full-length human cDNAs of androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), retinoic acid receptor alpha (RARα), and retinoic nuclear receptor alpha (RXRα).

Hybridization of the probes to RNA was carried out according to the method described by Church and Gilbert [45]. Blots were exposed to X-ray film for 30 days at −80°C.

RT-PCR

Primers were prepared at the local core facility (Center for Molecular Medicine) and purified by HPLC prior to use. cDNA was prepared using Superscript II Reverse Transcriptase (Gibco BRL) from total RNA of the following tissues and cells: colonic normal-appearing mucosa, human colonic fibroblasts, CD-Co 18, human intestinal smooth muscle cells, HISM, colonocytes isolated from surgical specimens of sigmoid mucosa, and colorectal carcinoma-derived cell lines, DLD-1, HT-29, SW 480, and SW 620. Total RNA isolated from LNCaP cells was used as a positive control for AR. Total RNA isolated from MCF-7 cells was used as a positive control for ER. Briefly, 2 μg of total RNA in 2 ml of DEPC water was mixed with 1 μl of 10 × DNase I buffer, 6 μl of DEPC water, and 1 μl of DNase I. The mixture was digested (25°C, 15 minutes) to remove any genomic DNA that might contaminate the RNA samples. DNase I was inactivated (1 μl 20 mM EDTA, 65°C, 10 minutes) and 1 μl oligo $(dT)_{12–18}$ (Gibco BRL, 500 μg/μl) was added. Subsequently, the samples were heated (70°C, 10 minutes) and quick-chilled on ice. After centrifugation, 4 μl of 5 × First Strand Buffer (Gibco BRL), 2 μl 0.1 M DTT, 1 μl 10mM dNTP, and 1 μl Superscript II Reverse Transcriptase (Gibco BRL) were added. The mixture was incubated for 1 hour at 37°C.

The PCR reaction mixtures contained 20 μl RT reaction product, 10 μl 10 × PCR buffer (Gibco BRL), 2 μl 10 mM dNTP, 3 μl 50mM $MgCl_2$, 1 μl sense primer (50 mM), 1 μl antisense primer (50 mM), 1 μl Taq DNA polymerase (Gibco BRL, 5U/ml), and sterile water to a final volume of 100 μl. PCR reaction mixtures were overlayed with 35 μl mineral oil in a 0.5 ml microfuge tube, then placed into a Perkin-Elmer thermocycler. Initially, samples were denatured at 95°C for 5 minutes, then amplified for 30 cycles of annealing for 1 minute at 52°C, elongation for 2.5 minute at 72°C, and denaturation for 1 minute 30 seconds at 95°C. The final elongation step was extended to 10 minutes for product completion. PCR reaction products were separated by electrophoresis on a 2% agarose gel at 60V for 2 hours, and the ethidium bromide-stained bands were visualized by UV transillumination. To confirm that the PCR products encode DNA binding domains of AR and ER, a separate RT-PCR reaction was performed according to the same protocol with additional 1 μl ^{32}P-dCTP at the PCR step. Sixteen μl of the radioactive product were digested with 1 μl Hind III, separately with 1 μl RSA I, run on the 10% polyacrylamide gel in 0.5 × TBE buffer at 900V for 2 hours, dried, and exposed to X-ray film. The enzymes were chosen on the base of the restriction map of the sequence encoding DNA binding domain of both receptors. In case of AR, Kpn I does not cut the 191 bp long fragment of DNA binding domain, and Rsa I cuts it at the position 104. In case of ER, no enzyme should cut the 205 bp long DNA binding domain. Negative controls for PCR omitted cDNA templates.

The sense AR sequence was 5′-CTG ATC TGT GGA GAT GAA GC. The antisense AR sequence was 5′-TTC CGA AGA CGA CAA GAT GG. The sense ER sequence was 5′-TGC AGT GTG CAA TGA CTA TGC. The antisense ER sequence was 5′-CAC TTC GTA GCA TTT GCG G. The sense β-Actin sequence was 5′-GCG CTC GTC GTC GAC AAC GGC. The antisense β-Actin sequence was 5′-CAT GGG GTA GCT CGT GCC GTA GCA GT.

RESULTS

Scatchard Analysis of Sex Steroid Receptors in Surgical Specimens

Figure 1A,B illustrates representative Scatchard plots for AR and ER proteins in the surgical specimens. These figures show results of the analysis of both receptors in normal-appearing mucosa and tumor of the same rectal cancer patient. The plots are linear, commonly regarded as an indication of a single class of binding sites.

Table I summarizes the results of Scatchard analyses of surgical specimens. AR protein was present at low amounts in all surgical specimens studied (n = 20) as demonstrated by plots similar to those shown in Figure 1A. Figure 1B shows the corresponding results for ER for the same patient and demonstrates the presence of ER protein. ER protein was identified in 10/13 normal colorectal mucosa specimens and 4/7 colorectal carcinomas at amounts even lower than was found for AR. The similarity in K_d values indicates that the same class of receptors is being measured in all the tissues. The B_{max} of both AR and ER in normal-appearing mucosa of control patients were similar to those found in normal-appearing mucosa specimens obtained from cancer patients.

Analysis of Sex Steroid Receptor Expression in Isolated Normal Colonocytes and Colorectal Carcinoma-derived Cell Lines

In contrast to the findings in tissues, no receptor proteins were identified by Scatchard analysis or ELISA assays in cytosol of colonocytes isolated from sigmoid

TABLE I. Mean Values of Dissociation Constant (K_d) and Maximal Binding Capacity (B_{max}) for Androgen Receptor (AR) and Estrogen Receptor (ER) as Measured by Scatchard Method in Normal Intestinal Tissues and Colorectal Carcinomas (CRC)*

	AR			ER			
		B_{max}			B_{max}		DNA
Tissue	K_d pmol/l	fmol/g tissue	fmol/mg DNA	Kd pmol/l	fmol/g tissue	fmol/mg DNA	mg/g tissue
NAM	278	121	42	206	38	13	2.97
	S.D. 244	S.D. 14	S.D. 6	S.D. 45	S.D. 12	S.D. 4	S.D. 0.7
TNAM	200	121	38	307	49	16	3.19
-2	S.D. 52	S.D. 18	S.D. 7	S.D. 74	S.D. 3	S.D. 1	S.D. 0.26
TNAM	343	132	39	285	51	15	3.55
-10	S.D. 184	S.D. 26	S.D. 13	S.D. 65	S.D. 7	S.D. 5	S.D. 0.58
CRC	514	88	19	237	86	19	4.74
	S.D. 263	S.D. 31	S.D. 7	S.D. 70	S.D. 39	S.D. 9	S.D. 0.71

*S.D. is the standard deviation. NAM and TNAM denote normal-appearing intestinal mucosa obtained from noncancer and cancer patients respectively. Suffixes -2 and -10 indicate the distance in cm from the primary tumor.

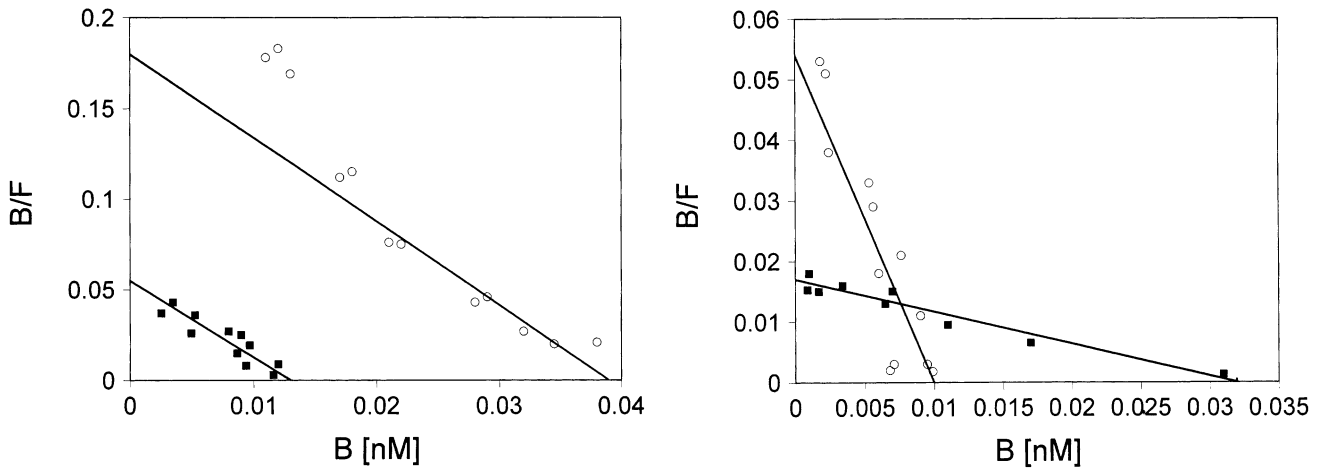

Fig. 1. Scatchard analysis of androgen and estrogen receptor for a single patient. **A.** Scatchard plot of methyltrienolone binding (androgen receptor) for one case of colorectal carcinoma cytosol (○) and to normal-appearing mucosa taken 10 cm from the tumor (■). **B.** Scatchard plots of beta estradiol binding for one case of colorectal carcinoma cytosol (○) and to normal-appearing mucosa taken 10 cm from the tumor (■). The data shown are the mean of duplicate determinations. All plots are linear, commonly regarded as an indication of a single class binding sites.

mucosa, DLD 1 cells, or HT 29 cells. As presented in the Materials and Methods section, these preparations consisted of 97% viable colonocytes without RBC, fibroblast, and smooth muscle cell contamination, with <2% contamination by lymphocytes. The distribution of points in the Scatchard plots was random in all three cases, the slope of the plot being zero within the 95% confidence interval. An example of a negative Scatchard analysis for AR and ER in colonocytes is shown in Figure 2B. The corresponding positive controls (LNCaP and MCF-7) support the negative findings. The absence of ER was confirmed by negative findings with ELISA.

Northern blotting with cDNA probes to AR and ER with mRNA isolated from populations of normal colonocytes and normal enterocytes and established cell lines (Caco-2, Colo205, DLD-1, HT-29, SW620, SW837, SW1463) were inconclusive in that no bands were seen except for the control LNCaP and MCF-7 cells (data not shown). These findings suggested that if the receptor mRNAs were expressed in intestinal epithelial cells, they were expressed at low abundance.

RT-PCR was used as a more sensitive assay for receptor mRNAs with results as shown in Figures 3 and 4. Figure 3A shows the results of probing for androgen receptor with primers for AR DNA binding domain and Figure 4A shows corresponding results for ER DNA binding domain in the same total RNA samples. Figure 3A shows that an RT-PCR product of the expected molecular weight was identified in cDNA sample derived from human colonic mucosa, human colonic fibroblasts, and human intestinal smooth muscle cells, as well as in control LNCaP cells. No product was present either in normal or transformed intestinal epithelial cell-derived cDNAs. As predicted from the sequence of the AR DNA binding domain,

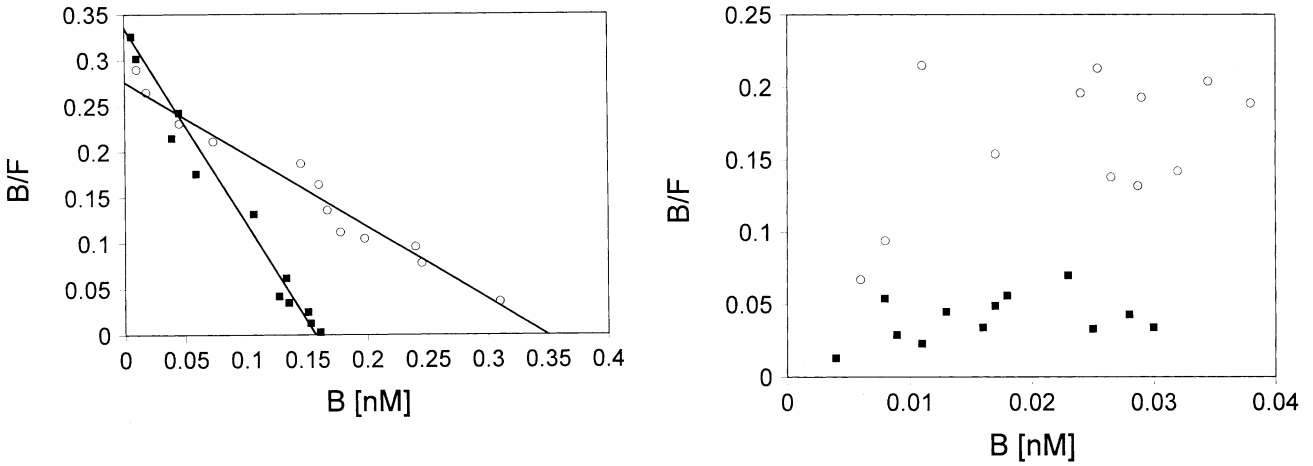

Fig. 2. Scatchard analysis of **(A)** androgen receptor in positive control cell line LNCaP (○) and estrogen receptor in positive control cell line MCF-7 (■), **(B)** androgen receptor (○) and estrogen receptor (■) in intestinal epithelial cells. The data shown are the mean of duplicate determinations. B illustrates a random distribution of points such as is obtained in the absence of receptor; the slope of the plots being zero within the 95% confidence interval.

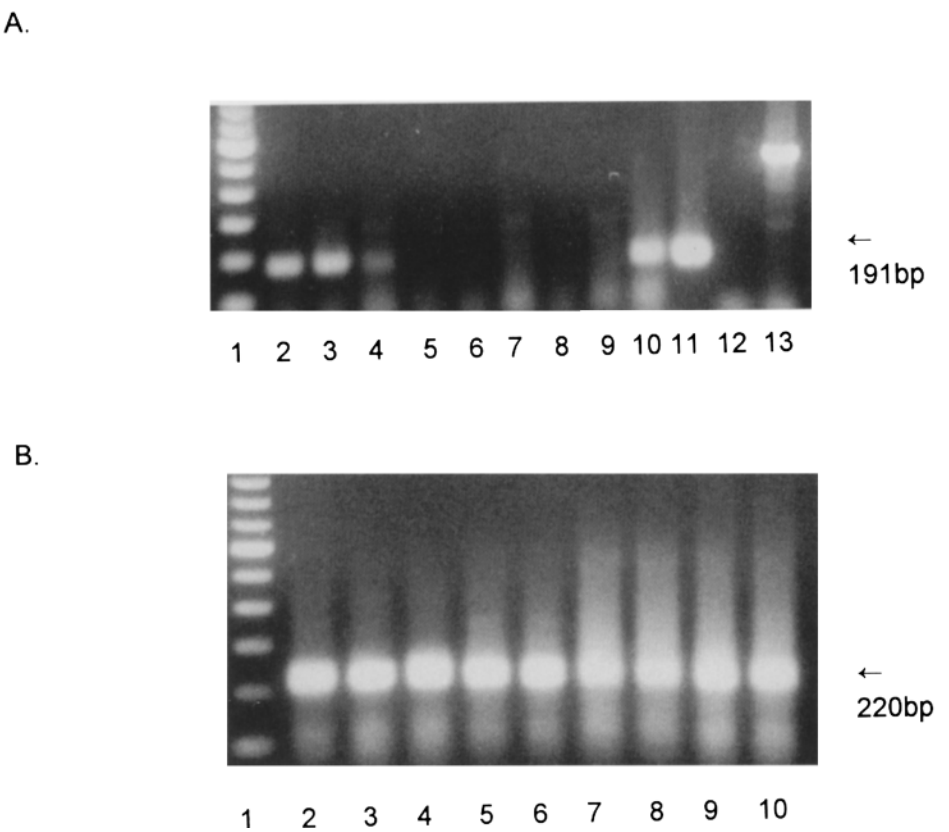

Fig. 3. Results of reverse transcription-polymerase chain reaction (RT-PCR) with primers for AR DNA binding domain **(A)** with corresponding β-actin RT-PCR products as controls **(B).** Lane 1 = DNA ladder at 100 bp interval; Lane 2 = human normal colonic mucosa; Lane 3 = human colonic fibroblasts; Lane 4 = human intestinal smooth muscle cells; Lane 5 = normal human colonocytes isolated from surgical specimens of sigmoid mucosa; Lane 6 = DLD-1 cell line; Lane 7 = HT-29 cell line; Lane 8 = SW480 cell line; Lane 9 = SW620 cell line; Lane 10 = LNCaP cell line (positive control); Lane 11 = human androgen receptor cDNA; Lane 12 = primers without template (negative control); Lane 13 = Perkin Elmer internal control for PCR kit. Arrow indicates RT-PCR product of the expected size.

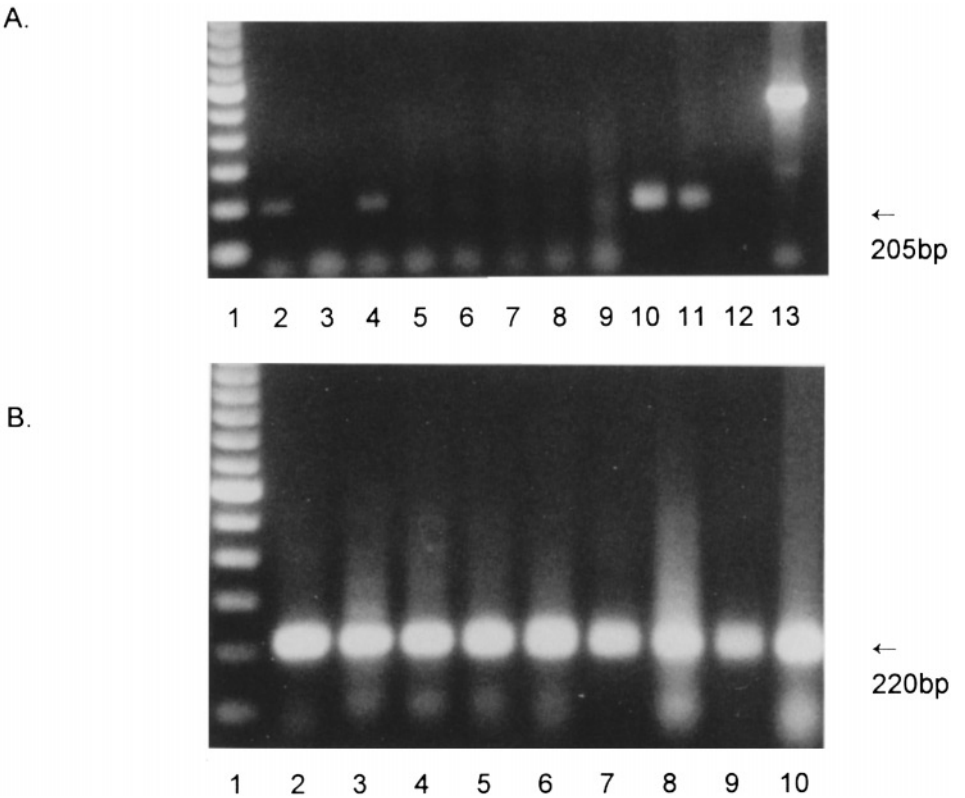

Fig. 4. Results of RT-PCR with primers for ER DNA binding domain **(A)** with corresponding β-actin RT-PCR products as controls **(B).** Lane 1 = DNA ladder at 100 bp interval; Lane 2 = human normal colonic mucosa; Lane 3 = human colonic fibroblasts; Lane 4 = human intestinal smooth muscle cells; Lane 5 = normal human colonocytes isolated from surgical specimens of sigmoid mucosa; Lane 6 = DLD-1 cell line; Lane 7 = HT-29 cell line; Lane 8 = SW480 cell line; Lane 9 = SW620 cell line; Lane 10 = MCF-7 cell line (positive control); Lane 1 = human estrogen receptor cDNA; Lane 12 = primers without template (negative control); Lane 13 = Perkin Elmer internal control for PCR kit. Arrow indicates RT-PCR product of the expected size.

restriction enzyme digestion of the PCR product with Kpn I gave no restriction fragments. Digestion with Rsa I gave two products of the expected size 104bp and 87bp (data not shown).

Figure 4A shows results of RT-PCR with primers for ER DNA binding domain. RT-PCR product corresponding to ER DNA binding domain was identified in cDNA sample derived from human colonic mucosa and human colonic smooth muscles cells only, and the control MCF-7 cells. As for AR, the identity of the band was confirmed from the pattern of restriction enzyme digestion with KpnI and Rsa I (data not shown).

DISCUSSION

Several previous studies have identified sex steroid receptors in surgical specimens of human colonic mucosa and primary colorectal carcinomas [3,22–28], but have not demonstrated which cells present in these tissues actually express the receptors. These findings have raised the question as to the function of sex steroids in bowel physiology. Differential rates of cancer in colon and rectum in females and males [46–49] suggest that sex-related functional differences exist and may relate to sex steroid signaling pathways. Our findings demonstrate the presence of sex steroid receptors in the stromal elements, but not in the intestinal epithelial cells. The observed distribution of receptors suggests that sex steroid effects on intestinal epithelium will relate to either stromal-epithelial interactions or to direct effects upon the cytoplasmic enzymes of epithelial cells.

Scatchard analysis proved that intestinal mucosa and colorectal cancer tissue contains sex steroid receptors. In order to avoid inaccuracies in AR measurement due to binding of the physiologic ligands, testosterone, or DHT to other proteins present in cytosol [35], the synthetic ligand methyltrienolone was used because of its higher affinity and specificity for the androgen receptor. AR and ER were identified in low, but detectable amounts in surgical specimens of both normal-appearing intestinal mucosa and in colorectal carcinomas. The amounts of AR in cancer tissues were consistently less than was seen in normal tissue, similar to previous findings [27]. ER was not apparently altered between normal and cancer tissues, but was not seen in three normal or three cancer specimens. The average K_d value of AR from cancer tissue was higher than was observed in the normal tissues,

but the large variance makes interpretation of the significance of the difference difficult. The difference in B_{max} is more clearcut. Two possibilities exist to explain the decrease in B_{max}. One is that the receptors are destroyed, due to lytic enzymes released during necrosis, or the low pH resulting from hypoxia. The other possibility is that the tumors have fewer stromal elements, and it is the stromal elements that express the sex steroid receptors.

When Scatchard analysis was applied to isolated cells, including normal colonocytes purified from tissue and colorectal carcinoma-derived cell lines, rather than whole mucosal tissue, the only lines that showed positive results were the control LNCaP and MCF-7 cells. Fibroblasts and smooth muscle cells from colon were not analyzed at this point, although the existence of sex steroid receptors in striated muscles and fibroblasts of other tissues had been reported [50,51]. An independent molecular technique was used to confirm the lack of expression of sex steroid receptor mRNAs in the intestinal epithelial cells and to investigate whether cells derived from the intestinal stroma expressed sex steroid receptor mRNAs. As shown in Figures 3 and 4, the results were clear-cut in demonstrating that only the cells derived from stroma and the control cells expressed sex steroid receptor mRNAs. It is unlikely that the patterns of expression represent artifacts. First, the Scatchard and RT-PCR analyses were positive for the appropriate control cells. Second, the same isolated colonocytes that did not express sex steroid receptor protein or mRNA did express hRARα protein and mRNA [4]. Without the demonstration of a lack of expression in the colonocytes isolated from patients, the results could have been ascribed to loss of expression of the receptors during culture. The results of the current study seem definitive on this point; colonocytes, either normal or transformed, do not express the sex steroid receptors.

Although many epithelial cell types examined to date seem to express sex steroid receptors, intestinal epithelial cells are an exception, at least in the mature individual. The function of the sex steroid receptors in the stromal cells of the intestinal mucosa is not known, but we speculate they may be involved in stromal regulation of proliferation and differentiation of intestinal epithelial cells, either normal or transformed. The putative effects of sex steroids would reside in stromal-epithelial interactions or direct effects upon cytoplasmic enzymes in intestinal epithelial cells. Reports of effects of sex steroids on colonic epithelium suggest both mechanisms may be operating [1,52,53].

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